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Translational genomics for better barley and beer

First cultivated more than 15,000 years ago, barley is used as a staple food, for animal feed, and for fermented beverages. Barley is the second most important cereal crop in Western Australia, delivering the state over $1 billion in export grain and malt earnings in recent years.

Developing the next generation of barley varieties with good flexibility and adaptability across different agricultural managements and environments is not easy, as the understanding of genetic controls for barley agronomic traits remains incomprehensive. The tribe of Triticeae plants including bread wheat, durum, rye and barley, has some of the most complex genetic histories among the world's cultivated species. The barley genome is 12 times larger than that of rice and almost twice the size of human genome. The major challenge in mapping the barley genome is due to the extreme abundance of repetitive elements and the severely reduced frequency of meiotic recombination in pericentromeric regions.

Recently, a reference barley genome sequence has been released in the frame of the International Barley Genome Sequencing Consortium and we mapped two of the seven chromosomes, 5H and 7H[1]. This achievement has made it easier to identify the key genes that control traits, such as yield, quality, disease resistance, and adaption to climate.

Here, we list a summary of the barley genome assembly and our current research advances in barley translational genomics.

1. The barley bible — reference genome sequence

Novel technologies such as Nano-technology, supercomputing, next-generation sequencing and chromosome conformation capture sequencing were integrated to generate a high-quality barley reference genome sequence. The reference sequence revealed why barley is selected for brewing. During malting, amylase proteins are produced in germinated seeds to decompose starch, yielding simple sugars for fermentation by yeast to produce alcohol. Compared with other cereal crops, there are 12 α-amylase family sequences in the genome of barley cultivar Morex. Gene duplication events have occurred in the subfamilies *amy1* and *amy2* located on chromosomes 6H and 7H, respectively. The high variability of the *amy1_1* gene in landraces and modern malting cultivars is remarkable.

2. From mapbook to GPS — the ocean of molecular markers

Using the reference genome sequence as a template, the alliance has mapped the genomes of key Australian barley varieties: Hindmarsh, La Trobe, Commander and Baudin (Figure 1). The in-depth analysis

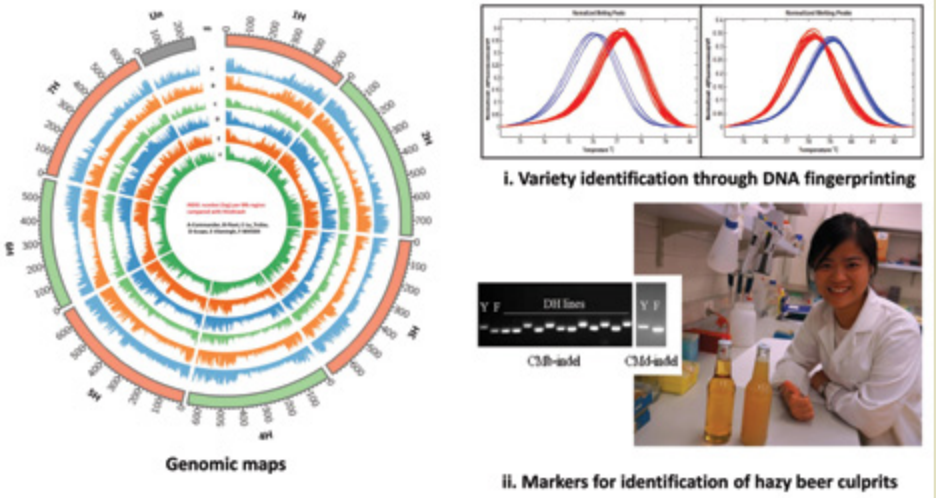


FIGURE 1 Genomic maps of Australian barley varieties with millions of molecular markers and some applications. We maintain strong links to grain and beer industries, such as CBH Ltd, Australian Grain Technologies (AGT), InterGrain, and Tsingtao Brewery through this work

FIGURE 2

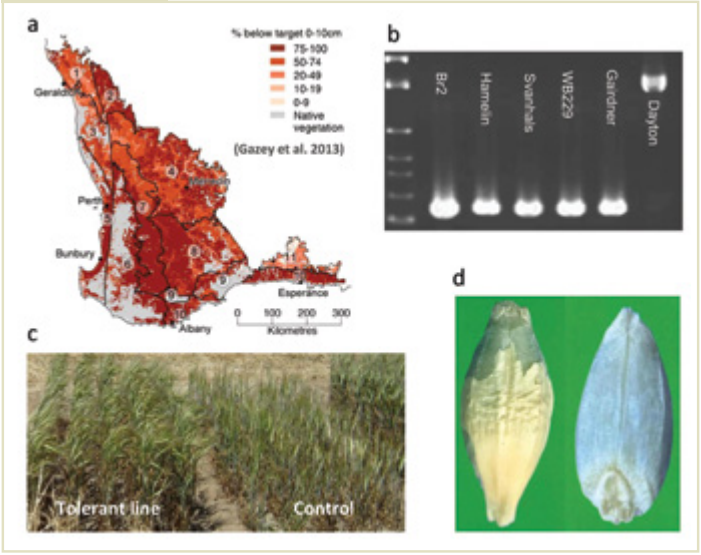
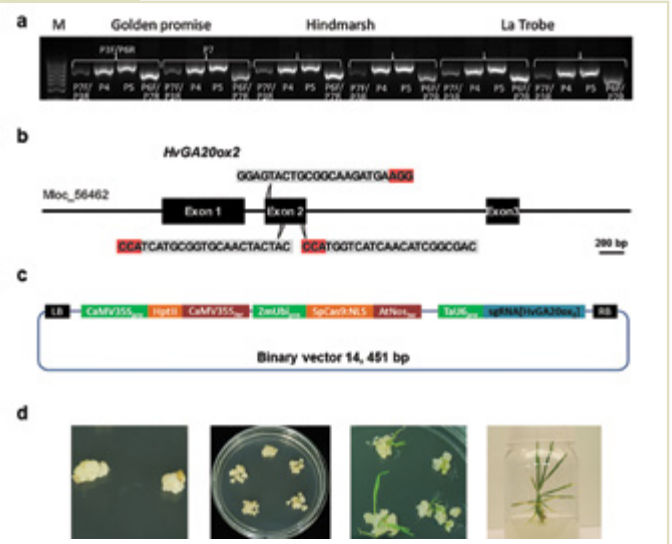


FIGURE 3



provides a comprehensive perspective of the genetic diversity among these germplasms, revealing millions of molecular markers including insertions and deletions (INDELs), single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs). At present, the coverage/density of markers has been greatly improved from average one marker for 40 genes to 50 markers per gene. The developed diagnostic markers could not only be used in targeting and marker-assisted selection for key agronomic traits for breeding programmes (i.e. hazy beer that cause a loss in flavour, foam quality and profit), but also in seed purity/identity test through DNA fingerprinting, which eventually contributes to the barley growers and enhances industry competitiveness.

3. White but not blue — new acid soil tolerant barley

Soil acidity is an economic and natural resource threat throughout the south-western agricultural area of Western Australia. More than 14.25 million hectares of wheatbelt soils are currently estimated to be acidic or at risk of becoming acidic to the point of restricting production. This damage results in a 9% production loss of annual cereal crops (Figure 2a). Screening barley germplasm for acid soil tolerance revealed significant genetic variation in this trait. The *HvMATE* gene conferring acid tolerance was identified through genetic mapping and two INDEL markers were also developed (Figure 2b). Based on a cross to the acid-tolerant line WB229, a new barley variety 'Litmus' was developed with outperforming yield potentials (Figure 2c). A blue colouring in the aleurone layer of Litmus grain has been detected (Figure 2d) that is closely linked to the *HvMATE* gene. Recently, the candidate gene controlling anthocyanin biosynthesis associated with the blue aleurone has been mapped and functionally characterised. Australia is committed to supplying customers of malt, food and feed grade barley with grain that only has a white aleurone, and this study will facilitate future breeding for acid-tolerant barley with white aluerone.

4. Making barley varieties better — genome editing technology

Genome editing is a genetic engineering approach where endogenous DNA is inserted, removed or replaced in the genome in a precise manner. Genome editing offers technical advantages over traditional genetic-modification (GM) techniques. The new emerging CRISPR-cas9 technology has been successfully used for major crops including wheat, rice, maize and canola. It has the potential to modify any traits and avoid the GM controversy. A technical platform for barley genome editing has been established (Figure 3) and now the technology could be introduced to commercial barley varieties. Apart from creating novel alleles or mutations in target genes, CRISPR-Cas9 genome editing could be used to alter genes to develop new traits in barley, such as stress tolerance and disease resistance.

FIGURE 2 Translate research to productivity — acid soil tolerant barley

FIGURE 3 Targeted gene mutation in *HvGA20ox2* using CRISPR-Cas9. **a**: full-length gene cloning from three barley cultivars through nested-PCR. **b**: *HvGA20ox2* gene model and target sequences for CRISPR-Cas9 genome editing. **c**: schematic of binary vector delivered to barley. **d**: *Agrobacterium*-mediated barley (cultivar 'Golden Promise') transformation

The Western Barley Genetics Alliance is successfully applying barley genetic information for variety identification through DNA fingerprinting, improving acid soil tolerance, and genome editing. ■

More information

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Reference

1 Mascher *et al*. 2017 A chromosome conformation capture ordered sequence of the barley genome. Nature 544: 427–433.